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(57) Abstract

A method for treating a retroviral infection in a cell, tissue or animal so-infected by administering a retrovirus replication inhibitory amount of nitric oxide or a nitric oxide-delivering, releasing or transferring compound. A method for preventing or reversing retroviral latency in a cell, tissue or animal by administering thereto a nitric oxide synthase inhibitor in an amount sufficient to render retrovirus replication-competent. A method for the treatment of a latent retrovirus infection in a cell, tissue or animal by administering (i) a nitric oxide synthase inhibitor or nitric oxide scavenger in an amount sufficient to render the retrovirus replication-competent and (ii) a replication inhibitory amount of an antiviral agent. A composition comprising (i) a nitric oxide synthase inhibitor or nitric oxide scavenger in an amount sufficient to render the retrovirus replication-competent and (ii) a replication inhibitory amount of an antiviral agent in a pharmaceutically acceptable carrier.

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INHIBITING RETROVIRAL REPLICATION

This invention relates to the field of inhibiting retroviral replication *in vitro* and *in vivo* and to the treatment in humans and other animals of active and latent retroviral infections.

The invention is based on the discovery by the inventors that nitric oxide and adducts thereof which are nitric oxide donors inhibit retroviral replication and provides a method for inhibiting the replication of a retrovirus by exposing the retrovirus to nitric oxide or a nitric oxide-releasing, donating or transferring substance.

Thus, in one aspect, the present invention provides a method for inhibiting retroviral replication in a retrovirally infected cell or tissue culture *in vitro* which comprises contacting said retrovirally infected cell or tissue culture with an amount of nitric oxide or a nitric oxide-delivering, releasing or transferring compound sufficient to inhibit retroviral replication in said retrovirally infected cell or tissue culture.

Another aspect of the invention provides a method for inhibiting retroviral replication in a retrovirally infected animal which comprises administering to said retrovirally infected animal an amount of nitric oxide or a nitric oxide-delivering, releasing or transferring compound sufficient to inhibit retroviral replication in said retrovirally infected animal.

Another aspect of the invention provides a method for inhibiting retroviral replication in a retrovirally infected individual which comprises administering to said retrovirally infected individual an amount of nitric oxide or a nitric oxide-delivering, releasing or transferring compound sufficient to inhibit retroviral replication in said retrovirally infected individual.

Another aspect of the invention provides a method for inhibiting the reactivation of latent retrovirus in a retrovirally infected cell or tissue culture which comprises contacting said retrovirally infected cell or tissue culture with a retrovirus reactivation inhibiting amount of nitric oxide, or a nitric oxide releasing, donating, or transferring substance which may be used prophylactically to prevent reactivation of latent retrovirus. This is very useful in mitigating the dangers of sample handling for research, laboratory and hospital personnel.

Another aspect of the invention provides a method for inhibiting the reactivation of a latent retrovirus infection in an animal in need thereof which comprises administering to said animal in need thereof an amount of nitric oxide, or a nitric oxide releasing, donating, or transferring substance sufficient to inhibit reactivation of latent retrovirus in said animal.

Another aspect of the invention provides a method for inhibiting the reactivation of a latent retrovirus infection in an individual in need thereof which comprises administering to an individual in need thereof an amount of nitric oxide, or a nitric oxide releasing, donating, or transferring substance sufficient to inhibit

reactivation of latent retrovirus in said individual. This is very useful, for example, in an immunocompromised host in which reactivation at an inopportune time (*e.g.* when defenses are low due to chemotherapy) would cause significant morbidity.

The invention is further based on the discovery by the inventors that nitric oxide synthase inhibitors and nitric oxide scavengers activate latent retrovirus. This is the first demonstration of a mechanism for eradicating the latent state, *i.e.*, once activated, the retrovirus can then be eliminated with antiviral therapy. Thus, this aspect of the present invention is directed to the use of nitric oxide synthase inhibitors as a means to eradicate latent virus.

Thus, another aspect of the invention provides a method for preventing or reversing retroviral latency in a retrovirally infected cell or tissue culture *in vitro* which comprises contacting a retrovirally infected cell or tissue culture *in vitro* with a nitric oxide synthase inhibitor or nitric oxide scavenger, such as hemoglobin, in an amount sufficient to render the latent retrovirus in the retrovirally infected cell or tissue culture replication-competent.

Another aspect of the invention provides a composition for the treatment of a latent retrovirus infection in an animal infected with a latent retrovirus infection which comprises (i) a nitric oxide synthase inhibitor or nitric oxide scavenger, such as hemoglobin, in an amount sufficient to render the retrovirus infecting said animal replication-competent and (ii) a retrovirus replication inhibitory amount of an antiviral agent in a pharmaceutically acceptable carrier.

Another aspect of the invention provides a method for the treatment of a latent retrovirus infection in an animal infected with a latent retrovirus infection which comprises administering thereto (i) a nitric oxide synthase inhibitor or nitric oxide scavenger, such as hemoglobin, in an amount sufficient to render the retrovirus

infecting said animal replication-competent and (ii) a retroviral replication inhibitory amount of an antiviral agent.

Another aspect of the invention provides a composition for the treatment of a latent retrovirus infection in an individual infected with a latent retrovirus infection which comprises (i) a nitric oxide synthase inhibitor or nitric oxide scavenger, such as hemoglobin, in an amount sufficient to render the retrovirus infecting said individual replication-competent and (ii) a retrovirus replication inhibitory amount of an antiviral agent in a pharmaceutically acceptable carrier.

Another aspect of the invention provides a method for the treatment of a latent retrovirus infection in an individual infected with a latent retrovirus infection which comprises administering thereto (i) a nitric oxide synthase inhibitor or nitric oxide scavenger, such as hemoglobin, in an amount sufficient to render the retrovirus infecting said individual replication-competent and (ii) a retroviral replication inhibitory amount of an antiviral agent.

Retroviruses whose growth may be inhibited in accordance with the method of the present invention include, but are not limited to, human immunodeficiency virus (HIV), human T-cell leukemia virus-1 (HTLV-1) and simian immunodeficiency virus (SIV). The administration can be topical, by inhalation, oral, or parenteral. The treated infection can be localized or systemic.

Figures 1A-1D show that NO generating compounds inhibit HIV-1 replication in human peripheral blood mononuclear cells and in the chronically infected human T cell line H9:

Figure 1A shows results when human peripheral blood mononuclear cells (PBMCs) stimulated with PHA and IL-2 were infected with HIV-1, and then grown in the presence or absence of the NO generating compound S-nitroso-N-acetyl-penicillamine (SNAP), or the parent compound N-acetyl-penicillamine (NAP):

Figure 1B shows the results when the same experiment as shown in Figure 1A was performed using the NO-generating compound S-nitroso-N-acetyl-cysteine (SNAC) or the parent compound N-acetyl cysteine (NAC);

Figure 1C shows the results when H9 cells were grown in the presence or absence of the NO generating compound SNAP; and

Figure 1D shows the results when the same experiment as shown in Figure 1C was performed using the NO generating compound S-nitroso-penicillamine (SNP) or the control compound penicillamine (P).

Figures 2A-2B show the effect of NO on PBMC proliferation:

Figure 2A shows the results when human peripheral blood mononuclear cells are stimulated with PHA and IL-2 were grown in the presence or absence of the NO-generating compound SNAP or the parent compound NAP; and

Figure 2B shows the results when the same experiment as shown in Figure 2A was performed using the NO-generating compound S-nitroso-N-acetyl cysteine (SNAC) or the control compound N-acetyl cysteine (NAC).

Figures 3A-3B show that iNOS is expressed constitutively in U-937 cells:

Figure 3A shows cDNA that was made from whole cell RNA from U-937 or from the control B cell line B-958 which is known to express iNOS; and

Figure 3B shows a western blot that was performed on whole cell lysates made from 0.5×10^6 U-937 cells (U-937), the human B cell line BL-30, and rat macrophages stimulated with LPS (Rat).

Figures 4A-4B show that endogenously produced NO inhibits HIV replication and HIV reactivation:

Figure 4A shows the results when U-937 cells were infected with HIV-1 and then grown in the presence or absence of the NOS inhibitor L-NMA; and

Figure 4B shows the results when the latently infected U1 cell line was grown in the presence or absence of L-NMA and TNFα.

Figure 5 shows the effect of cGMP on HIV replication. Peripheral blood mononuclear cells were infected with HIV-1 and then grown in the presence or absence of SNAP, NAP or the cGMP analog 8-bromo-cGMP.

Compounds contemplated for use in the invention are nitric oxide and compounds that release nitric oxide or otherwise directly or indirectly deliver or transfer nitric oxide to a site of its activity, such as on a cell membrane, *in vivo*. As used here, the term "nitric oxide" encompasses uncharged nitric oxide(NO•) and charged nitric oxide species, particularly including nitrosonium ion(NO^+) and nitroxyl ion(NO^-). The reactive form of nitric oxide can be provided by gaseous nitric oxide. The nitric oxide releasing, delivering or transferring compounds, having the structure X-NO wherein X is a nitric oxide releasing, delivering or transferring moiety, include any and all such compounds which provide nitric oxide to its intended site of action in a form active for their intended purpose. As used here, the term "NO adducts" encompasses any of such nitric oxide releasing, delivering or transferring compounds, including, for example, S-nitrosothiols, S-nitroso amino acids, S-nitroso-polypeptides, and nitrosoamines. It is contemplated that any or all of these "NO adducts can be mono- or poly- nirosylated at a variety of naturally susceptible or artificially provided binding sites for nitric oxide,

One group of such NO adducts is the S-nitrosothiols, which are compounds that include at least one -S-NO group. Such compounds include S-nitroso-polypeptides (the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function, and derivatives thereof): S-nitrosylated amino acids(including natural and synthetic amino acids and their stereoisomers and racemic mixtures and derivatives thereof); S-nitrosated sugars, S-nitrosated-modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200, nucleotides): and an S-nitrosated hydrocarbon where the hydrocarbon can be a branched or unbranched, and saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon: S-nitroso hydrocarbons having one or

more substituent groups in addition to the S-nitroso group: and heterocyclic compounds. S-nitrosothiols and the methods for preparing them are described in U.S. Patent Application No. 07/943,834, filed September 14, 1992, Oae *et al.*, Org. Prep. Proc. Int., 15(3):165-198 (1983); Loscalzo *et al.*, J. Pharmacol. Exp. Ther., 249(3):726729 (1989) and Kowaluk *et al.*, J. Pharmacol. Exp. Ther., 256:1256-1264 (1990), all of which are incorporated in their entirety by reference.

One particularly preferred embodiment of this aspect relates to S-nitroso amino acids where the nitroso group is linked to a sulfur group of a sulfur-containing amino acid or derivative thereof. For example, such compounds include the following: S-nitroso-N-acetylcysteine, S-nitroso-captopril, S-nitroso-homocysteine, S-nitroso-cysteine and S-nitroso-glutathione.

Suitable S-nitrosylated proteins include thiol-containing proteins(where the NO group is attached to one or more sulfur group on an amino acid or amino acid derivative thereof) from various functional classes including enzymes, such as tissue-type plasminogen activator(TPA) and cathepsin B; transport proteins, such as lipoproteins, heme proteins such as hemoglobin and serum albumin; and biologically protective proteins, such as the immunoglobulins and the cytokines. Such nitrosylated proteins are described in PCT Publ. Applic. No. WO 93/09806, published May 27, 1993. Examples include polynitrosylated albumin where multiple thiol or other nucleophilic centers in the protein are modified.

Further examples of suitable S-nitrosothiols include those having the structures:

(i) $CH_3(CH_2)_xSNO$

wherein x equals 2 to 20;

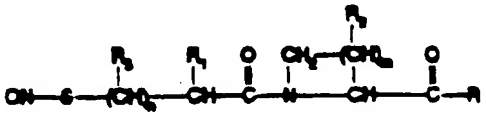
(ii) $HS(CH_2)_xSNO$

wherein x equals 2 to 20; and

(iii) $ONS(CH_2)_xY$

wherein x equals 2 to 20 and Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl; wherein aryl includes benzyl, naphthyl, and anthracenyl groups.

Other suitable S-nitrosothiols that are S-nitroso-angiotensin converting enzyme inhibitors (hereinafter referred to as S-nitroso-ACE inhibitors) are described in Loscalzo, U.S. Patent No. 5,002,964 (1991) and Loscalzo *et al.*, U.S. Patent No. 5,025,001 (1991) both of which are incorporated in their entirety by reference. Examples of such S-nitroso-ACE inhibitors include compounds having structure (1):



(1)

wherein

R is hydroxy, NH_2, NHR^4, NR^4R^5, or C_1-C_7 alkoxy, wherein R^4 and R^5 are C_1-C_4 alkyl, or phenyl, or C_1-C_4 alkyl substituted by phenyl;

R^1 is hydrogen, C_1-C_7 alkyl, or C_1-C_7 alkyl substituted by phenyl, amino, guanidino, NHR^6, NR^6R^7, wherein R^6 and R^7 are methyl or C_1-C_4 alkanoyl;

R^2 is hydrogen, hydroxy, C_1-C_4 alkoxy, phenoxy, or C_1-C_7 alkyl;

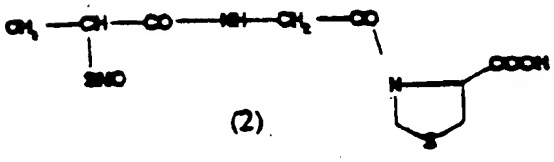
R^3 is hydrogen, C_1-C_4 or C_1-C_7 alkyl substituted by phenyl;

m is 1 to 3; and

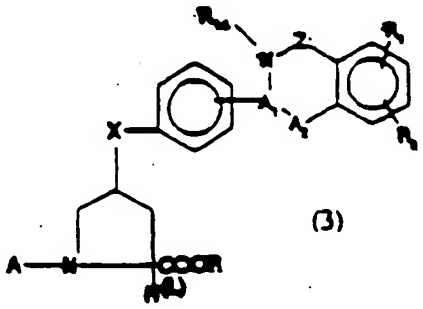
n is 0 to 2.

Other suitable S-nitroso-ACE inhibitors include N-acetyl-S-nitroso-D--cysteinyl-L-proline, N-acetyl-S-nitroso-D,L-cysteinyl-L-proline, 1-[4-amino-2-(S-nitroso)mercaptomethyl butanoyl]-L-proline; 1-[2-hexanoyl]-L-proline, 1-[5--guanidino-2-(S-nitroso)mercaptomethyl-pentanoyl]-L-proline, 1-[5-amino-2-(S-nitroso) mercaptomethyl-pentanoyl]-4-hydroxy-L-proline, 1-[5-guanidino-2-(S-nitroso)mercaptomethyl-pentanoyl]-4-hydroxy-L-proline, 1-[2-aminomethyl-3(S-nitroso)-mercaptomethyl-pentanoyl-L-proline, and S-nitroso-L-cysteinyl-L-proline.

Additional suitable S-nitroso-ACE inhibitors include those having structures (2-3):



(2)



(3)

wherein

X is oxygen or sulfur;

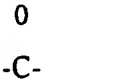
$-A_1-A_2-$ is CH-NH or -C=N-;

R_3 0

A is ON-S-CH_2-CH-C;

R is selected from hydrogen, lower (C_1-C_4) alkyl, benzyl, benzhydryl, and salt forming ion;

R_1 and R_2 are independently selected from hydrogen, halogen, lower alkyl, lower alkoxy, halo substituted lower alkyl, nitro, and SO_2NH_2;

Z is  or 

R_3 is hydrogen, lower alkyl, halo substituted lower alkyl, phenyl, benzyl, phenethyl, or cycloalkyl; and

R_4 is hydrogen, lower alkyl, halo substituted lower alkyl, hydroxy substituted lower alkyl, $-(CH_2)_q-N$ (lower alkyl)$_2$ or $-(CH_2)_q-NH_2$ and q is one, two, three or four.

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The S-nitroso-ACE inhibitors can be prepared by various methods of synthesis. In general, the thiol precursor is prepared first, then converted to the S-nitrosothiol derivative by nitrosation of the thiol group with $NaNO_2$ under acidic conditions (pH = 1 to 5) which yields the S-nitroso derivative. Acids which may be used for this purpose include aqueous sulfuric, acetic and hydrochloric acids. Thiol precursors are prepared as described in the following: U.S. Pat. Nos. 4,046,889 (1977); 4,052,511; 4,053,651; 4,113,751, 4,154,840, 4129,571 (1978), and 4,154,960 (1979) to Ondetti *et al.*; U.S. Pat. No. 4,626,545 (1986) to Taub; and U.S. Pat. Nos. 4,692,458 (1987) and 4,692,459 (1987) to Ryan *et al.*, Quadro, U.S. Pat. No. 4,447,419 (1984); Haugwitz *et al.*; U.S. Pat. No. 4,681,886 (1987), Bush *et al.*, U.S. Pat. No. 4,568,675 (1986), Bennion *et al.*, U.S. Pat. No. 4,748,160 (1988), Portlock, U.S. Pat. No. 4,461,896 (1984), Hoefle *et al.*, European Patent Application Publication No. 0 088 341 (1983), Huange *et al.*, U.S. Pat. No. 4,585,758 (1986), European Patent application Publication No. 0 237 239, European Patent application Publication No. 0 174 162, published in 1986, European Patent application Publication No. 0 257 485, published in 1988, all of which are incorporated by reference herein.

Another group of such NO adducts are compounds that include at least one -O-NO group. Such compounds include O-nitroso-polypeptides(the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function, and derivatives thereof); O-nitrosylated amino acids (including natural and synthetic amino acids and their stereoisomers and racemic mixtures and derivatives thereof); O-nitrosated sugars; O-nitrosated-modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200, nucleotides); and an O-nitrosated hydrocarbon where the hydrocarbon can be a branched or unbranched, saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon; O-nitroso hydrocarbons having one or more substituent groups in addition to the O-nitroso group; and heterocyclic compounds.

Another group of such NO adducts is the nitrites which have an -O-NO group wherein R is a protein, polypeptide, amino acid, branched or unbranched and saturated or unsaturated alkyl, aryl or a heterocyclic. A preferred example is the nitosylated form of isosorbide. Compounds in this group form S-nitrosothiol intermediates *in vivo* in the recipient human or other animal to be treated and can therefore include any structurally analogous precursor R-O-NO of the S-nitrosothiols described above.

Another group of such NO adducts is the N-nitrosoamines, which are compounds that include at least one -N-NO group. Such compounds include N-nitroso-polypeptides (the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function, and derivatives thereof); N-nitrosylated amino acids (including natural and synthetic amino acids and their stereoisomers and racemic mixtures); N-nitrosated sugars; N-nitrosated-modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200, nucleotides); and an N-nitrosated hydrocarbon where the hydrocarbon can be a branched or unbranched, and saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon; N-nitroso hydrocarbons having one or more substituent groups in addition to the N-nitroso group; and heterocyclic compounds.

Another group of such NO adducts is the C-nitroso compounds that include at least one -C-NO group. Such compounds include C-nitroso-polypeptides (the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function, and derivatives thereof); C-nitrosylated amino acids (including natural and synthetic amino acids and their stereoisomers and racemic mixtures); C-nitrosated sugars; C-nitrosated-modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200, nucleotides); and a C-nitrosated hydrocarbon where the hydrocarbon can be a branched or unbranched, and saturated or unsaturated aliphatic hydrocarbon, or an aromatic

hydrocarbon: C-nitroso hydrocarbons having one or more substituent groups in addition to the C-nitroso group: and heterocyclic compounds.

Another group of such NO adducts is the nitrates which have at least one -O-NO_2 group. Such compounds include polypeptides (the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function. and derivatives thereof): amino acids (including natural and synthetic amino acids and their stereoisomers and racemic mixtures and derivatives thereof): sugars: modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200. nucleotides): and a hydrocarbon where the hydrocarbon can be a branched or unbranched. and saturated or unsaturated aliphatic hydrocarbon. or an aromatic hydrocarbon: hydrocarbons having one or more substituent groups: and heterocyclic compounds. A preferred example is nitroglycerin.

Another group of such NO adducts is the nitroso-metal compounds which have the structure $(R)_n$-A-M-$(NO)_x$. R includes polypeptides(the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function. and derivatives thereof): amino acids (including natural and synthetic amino acids and their stereoisomers and racemic mixtures and derivatives thereof): sugars: modified and unmodified oligonucleotides (preferably of at least 5. and more particularly 5-200, nucleotides); and a hydrocarbon where the hydrocarbon can be a branched or unbranched. and saturated or unsaturated aliphatic hydrocarbon. or an aromatic hydrocarbon: hydrocarbons having one or more substituent groups in addition to the A-nitroso group; and heterocyclic compounds. A is S. O. or N. n and x are each integers independently selected from 1. 2 and 3. and M is a metal. preferably a transition metal. Preferred metals include iron. copper. manganese. cobalt. selenium and luthidium. Also contemplated are N-nitrosylated metal centers such as nitroprusside.

Another group of such NO adducts is the N-oxo-N- nitrosoamines which have an R-N(O$^-$M$^+$)-NO group or an R-NO-NO-group. R includes polypeptides (the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function, and derivatives thereof); amino acids(including natural and synthetic amino acids and their stereoisomers and racemic mixtures and derivatives thereof); sugars; modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200, nucleotides); and a hydrocarbon where the hydrocarbon can be a branched or unbranched, and saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon; hydrocarbons having one or more substituent groups; and heterocyclic compounds. R is preferably a nucleophilic (basic) moiety. M+ is a metal cation, such as, for example, a Group I metal cation.

Another group of such NO adducts is the thionitrates which have the structure R-(S)$_x$-NO wherein x is an integer of at least 2. R is as described above for the S-nitrosothiols. Preferred are the dithiols wherein x is 2. Particularly preferred are those compounds where R is a polypeptide or hydrocarbon and a par or pairs of thiols are sufficiently structurally proximate, *i.e.* vicinal, that the pair of thiols will be reduced to a disulfide. Those compounds which form disulfide species release nitroxyl ion(NO$^-$) and uncharged nitric oxide(NO●). Those compounds where the thiol groups are not sufficiently close to form disulfide bridges generally only provide nitric oxide as the NO$^-$ form but not as the uncharged NO● form.

Agents which stimulate endogenous NO synthesis are also suitable for use in accordance with the invention.

Viruses whose replication may be inhibited or prevented by the compounds described hereinabove include retroviruses such as human immunodeficiency virus (HIV), human T-cell leukemia virus-I (HTLV-1), and simian immunodeficiency virus (SIV). Thus, these compounds can be employed in various therapeutic treatments, including: 1) the incorporation of NO donors into creams, salves or

lotions for the treatment of topical infections: 2) the use of inhaled nitric oxide and other NO donors for the treatment of lung infections: 3) the use of topical drops and creams for the treatment of mucocutaneous membrane infections, such as infections of the eye, mouth and genitourinary tract: and 4) oral or parenteral administration for systemic or localized infections.

When administered *in vivo*, the nitric oxide may be administered in combination with pharmaceutical carriers and in dosages described herein.

Certain viruses can both replicate (lytic phase) and also incorporate themselves into the genome (latency). One associates infectious disease with the replicative cycle of the virus; however, significant morbidity may be associated with the latent phase as well. Further, certain viruses tend to establish latency more readily than others. When latent, the virus may serve as a protooncogene, thereby promoting cancer: and it is also resistant to antiviral therapy. Thus, the establishment of latency prevents elimination of virus with antiviral therapy.

Another aspect of the invention is based on the discovery that nitric oxide synthase inhibitors activate latent retrovirus. This is the first demonstration of a mechanism for eradicating the latent state, i.e., once activated, the retrovirus can then be eliminated with antiviral therapy. Thus, this aspect of the present invention is directed to the use of nitric oxide synthase inhibitors as a means to eradicate latent virus.

Thus, in one embodiment of this aspect, the invention provides a method for preventing or reversing retroviral latency in a cell, tissue or animal infected therewith which comprises administering thereto a nitric oxide synthase inhibitor in an amount sufficient to render the retrovirus replication-competent.

In another embodiment, this aspect of the invention provides a method for the treatment of a latent retrovirus infection in a cell, tissue or animal so-infected, which comprises administering thereto (i) a nitric oxide synthase inhibitor in an amount sufficient to render the retrovirus replication-competent and (ii) a replication inhibitory amount of an antiviral agent.

In another embodiment, this aspect of the invention provides a composition for the treatment of a latent retrovirus infection in a cell, tissue or animal so-infected, which comprises (i) a nitric oxide synthase inhibitor in an amount sufficient to render the retrovirus replication-competent and (ii) a replication inhibitory amount of an antiviral agent in a pharmaceutically acceptable carrier.

Suitable nitric oxide synthase inhibitors include arginine derivatives such as N^G-monomethyl-L-arginine (NMA), nitro-arginine, diphenylene iodonium and related iodonium derivatives, N-nitro-L-arginine methyl ester, N-methyl-L-arginine, N-amino-L-arginine, ornithine, N-imino-ethyl-L-ornithine and ornithine derivatives, N-nitro-L-arginine, methylene blue, heme binders, trifluoropiperazine, and calcinarin and other calmodulin binders, such as methotrexate.

The term "antiviral agent," refers to any agent that inhibits, prevents, or destroys the growth or replication of a retrovirus. The antiviral agent can be a purine nucleoside analog such as acyclovir or didanosine. Alternatively, the antiviral agent can be a a pyrimidine nucleoside analog such as zidovudine, trifluridine and zalcitabine. The antiviral agent can also be a chelating agent such as foscarnet sodium. The antiviral agent can be an intercalating agent. Further, the antiviral agent can be an interferon, preferably an α-interferon, a β-interferon or a γ-interferon. Other known antiviral agents and those subsequently to be discovered are contemplated as within the scope of this term.

Treatment of localized topical infection with the nitric oxide or nitric oxide releasing agent comprises contacting the surface so as to cause the surface to be coated with the nitric oxide or nitric oxide releasing agent. Coating may be accomplished using standard methods well known to those of ordinary skill in the art. For example, coating a surface with nitric oxide adducts can be achieved by bathing the surface in a solution containing the nitric oxide adduct. In addition, synthetic nitric oxide adducts may be coated onto an artificial surface such as a bandage or covering by a variety of chemical techniques which are well known in the art.

Modes of administration include but are not limited to topical, transdermal, intramuscular, intraperitoneal, intravenous, vaginal, subcutaneous, intranasal, topical and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents.

Principal approaches to therapeutic uses of the invention include topical administration to treat skin infections; topical administration to treat genital and oral lesions; topical administration to treat eye infections; topical or inhalation administration of NO/or SNAC to treat respiratory infections; and particularly intravenous administration to treat systemic infection.

The compounds of this invention can be employed in combination with conventional excipients, i. e., pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral application which do not deleteriously react with the active compounds. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohol, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, petroethral fatty acid esters, hydroxymethylcellulose, polyvinylpyrrolidone, etc. The pharmaceutical

preparations can be sterilized and if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds. For parenteral application, particularly suitable vehicles consist of solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants. Aqueous suspensions may contain substances which increase the viscosity of the suspension and include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

Various delivery systems are known and can be used to administer a therapeutic compound or composition of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules and the like.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ameliorate any pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a drylyophilized powder or water free concentrate in a hermetically sealed container such as an

ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, sulfuric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The term "therapeutically effective amount," for the purposes of the invention, refers to the amount of the nitric oxide adduct which is effective to achieve its intended purpose. While individual needs vary, determination of optimal ranges for effective amounts of each nitric oxide adduct is within the skill of the art. Generally, the dosage required to provide an effective amount of the composition, and which can be adjusted by one of ordinary skill in the art will vary, depending on the age, health, physical condition, sex, weight, extent of disease of the recipient, frequency of treatment and the nature and scope of the disorder.

The amount of the therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage

ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems and are in the same ranges or less than as described for the commercially available compounds in the Physician's Desk Reference, Medical Economics Company, Inc., Oradell, N.J. 1990.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

The following non-limitative examples further describe and enable one of ordinary skill in the art to make and use the invention.

EXAMPLE 1

In this example, the following materials were employed and experimental procedures were followed:

Infection of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells isolated from HIV-1 negative adult donors were stimulated for 48 hours with 10 μg/ml of PHA. The cells were then infected with HIV-1 strain MN at an MOI of 0.1 for 1-2 hours at 37°C, washed three times and resuspended at 500,000 cells/ml in complete medium (RPMI 1640 10% fetal bovine serum) supplemented with 10 units/ml of IL-10 with or without various concentrations of SNAP, NAP, SNAC or NAC. The cells were then plated in 24

well plates with 2 ml of cells per well. After 4 days, the cells were split and refed with the same media in which they were originally resuspended. After 5-8 days in culture, cell supernatants were collected, and p24 antigen levels determined using the HIV-1 p24 Core Profile assay (NEN) as per the manufacturer's instructions.

Infection of H9 cells

H9 cells were pelleted and infected with HIV-1 strain MN at an MOI of 0.1 for 1-2 hours at 37°C, washed three times, resuspended at a concentration of 100,000 cells/ml in complete medium supplemented with or without SNAP, NAP, SNOP or penicillamine, and plated in 24 well plates with 2 ml of cells per well. After 3 days, p24 antigen levels were measured in the H9 cell supernatants using the method described above.

Infection of U937 cells

U937 cells were pelleted and infected with HIV-1 strain MN at an MOI of 0.2 for 1-2 hours at 37°C, washed three times and then resuspended at 100,000 cells/ml in RPMI 1640 containing 10% FBS and 0.1 mM arginine with or without L-NMA. The cells were then plated in 24 well plates containing 2 ml of cells per well. Twice a week the cells were split and refed with the same media in which they were originally resuspended. After 8-14 days, p24 antigen levels were measured in the cell supernatants using the method described above.

Reactivation of virus from U1 cells

U1 cells were resuspended at a concentration of 100,000 cells per ml in RPMI 1640 (GIBCO) containing 10% FBS and 0.1 mM arginine with or without TNF-α or L-NMA. The cells were then plated in 24 well plates containing 2 ml of cells per well. After 48-72 hours in culture, p24 antigen levels were measured in the cell supernatants using the method described above.

The effect of NO generating compounds on HIV-1 replication was determined by growing human peripheral blood mononuclear cells (PBMCs) infected with HIV-1 (MN strain) in the presence or absence of NO-generating compounds or control parent compounds which do not generate NO. The extent of HIV-1 replication was determined 5 to 8 days after infection by measuring p24 antigen levels in the cell supernatants.

Figures 1A-1D show that NO generating compounds inhibit HIV-1 replication in human peripheral blood mononuclear cells and in the chronically infected human T cell line H9.

Figure 1A shows results when human peripheral blood mononuclear cells (PBMCs) stimulated with PHA and IL-2 were infected with HIV-1, and then grown in the presence or absence of the NO generating compound S-nitroso-N-acetyl-penicillamine (SNAP), or the parent compound N-acetyl-penicillamine (NAP). P24 levels were measured in the cell supernatants by ELISA 6 days after infection. As a control, cells were infected in the absence of added drug. The NO generating compound S-nitroso-N-acetyl-penicillamine (SNAP), but not the control parent compound N-acetyl-penicillamine (NAP), inhibited HIV replication in PBMCs in a dose dependent manner. The data are expressed as the percent of control p24 levels. Since SNAP and NAP were diluted in ETOH, the effect of 0.2% ETOH (the concentration present in 200 uM SNAP and NAP cultures) was determined. The results represent the mean of 9 separate experiments $\pm$ SEM for 100, 200, and 300 uM SNAP or NAP, and the mean of 2 separate experiments for 10, 50, 150 and 250 uM SNAP or NAP concentrations.

Figure 1B shows the results when the same experiment as shown in Figure 1A was performed using the NO-generating compound S-nitroso-N-acetyl-cysteine (SNAC) or the parent compound N-acetyl cysteine (NAC). Similar effects were seen with the NO-generating compound S-nitroso-N-acetyl-cysteine (SNAC) and the

control parent compound N-acetyl-cysteine (NAC). The data are expressed as the mean of duplicate cultures.

Figure 1C shows the results when H9 cells were grown in the presence or absence of the NO generating compound SNAP; and After three days in the culture. p24 levels in the cell supernatants were measured by ELISA. Again. the NO donor SNAP inhibited HIV replication in a dose dependent fashion. The data are expressed as the mean of duplicate cultures and are repesentative of one of three experiments.

Figure 1D shows the results when the same experiment as shown in Figure 1C was performed using the NO generating compound S-nitroso-penicillamine (SNP) or the control compound penicillamine (P). Similar results were seen with the NO donor S-nitroso-penicillamine (SNP) but not with the parent compound penicillamine (P). The data are expressed as the mean of duplicate cultures.

Since NO inhibits lymphocyte proliferation and inhibition of cellular proliferation is associated with an inhibition of HIV replication. we determined whether the SNAP-induced inhibition of HIV replication was due simply to an anti-proliferative effect of the compound. Figures 2A-2B show the effect of NO on PBMC proliferation.

Figure 2A shows the results when human peripheral blood mononuclear cells stimulated with PHA and IL-2 were grown in the presence or absence of the NO-generating compound SNAP or the parent compound NAP. The effects of SNAP or NAP on PBMC proliferation were analyzed by tritiated thymidine uptake. Higher concentrations of SNAP (i.e. 200 uM-500 uM) inhibited PBMC proliferation. however. 50-150 uM SNAP did not inhibit PBMC proliferation and yet inhibited p24 antigen production (Figure 1A). After 6 days in culture, [^{3}H]-thymidine uptake was measured. SNAP and NAP were diluted in ETOH and therefore. as a control. the effect of ETOH on PBMC proliferation was measured. The concentrations of ETOH

present in 100, 200, 300, and 400 uM SNAP or NAP were 0.1%, 0.2%, 0.3%, and 0.4% ETOH, respectively.

Figure 2B shows the results when the same experiment as shown in Figure 2A was performed using the NO-generating compound S-nitroso-N-acetyl cysteine (SNAC) or the control compound N-acetyl cysteine (NAC). After 6 days in culture the number of viable cells was determined by trypan blue staining. Concentrations of SNAC which inhibited HIV replication (Figure 1B) had no effect on PBMC proliferation. Thus NO appears to have an inhibitory effect on HIV replication which is independent of its anti-proliferative effect.

Since NO generating compounds inhibit HIV-1 replication, the effect of endogenously synthesized NO on HIV-1 replication was analyzed. NO synthase (NOS) activity is difficult to demonstrate in human mononuclear cells, presumably because they express very low levels of NOS. However, inducible NOS(iNOS) expression and activity was evident in the U-937 human promonocytic cell line. Figures 3A-3B show that iNOS is expressed constitutively in U-937 cells.

Figure 3A shows cDNA that was made from whole cell RNA from U-937 or from the control B cell line B-958 which is known to express iNOS. Samples to which reverse transcriptase was (+RT) or was not (-RT) added are indicated. Equal amounts of each sample were PCR amplified using primers specific for human iNOS. The markers on the left indicate the base pair size of OX DNA fragments generated from an Hae III digest. iNOS RNA expression was detected in U-937 cells by RT-PCR using human iNOS-specific primers. This RT-PCR product was sequenced and found to be identical to the human hepatic iNOS cDNA sequence.

Figure 3B shows a western blot that was performed on whole cell lysates made from 0.5×10^6 U-937 cells (U-937), the human B cell line BL-30, and rat macrophages stimulated with LPS (Rat). INOS expression was detected using an

affinity purified rabbit antiserum was raised against the C-terminal 20 amino acids of the rat isoform and recognizes both human and rat iNOS (Kobzik *et al.* 1993). The rat isoform, which shares 80% homology with the human isoform, runs slightly below the human isoform on polyacrylamide gels. In addition NO production by U-937 cells was detected by a photolysis/chemilumincesence assay.

This cell line, therefore, was chosen to study the effects of endogenously synthesized NO on HIV replication. U-937 cells were infected with HIV-1 (MN strain) at an MOI of 0.1, washed, and then grown in the presence or absence of N^G-monomethyl-L-arginine (L-NMA), a specific competitive inhibitor of NOS. Ten to fourteen days after infection, p24 levels in the cell supernatant were determined by ELISA. Figures 4A-4B show that endogenously produced NO inhibits HIV replication and HIV reactivation.

Figure 4A shows the results when U-937 cells were infected with HIV-1 and then grown in the presence or absence of the NOS inhibitor L-NMA; and After 8-10 days in culture, p24 levels in the cell supernatants were determined by ELISA. Inhibition of endogenous NO synthesis by L-NMA caused a small but statistically significant increase in p24 antigen levels, with maximal effects seen between 100-250 uM L-NMA. The L-NMA-induced increase in p24 levels was abrogated by the addition of excess L-arginine, the NOS substrate. Thus, both endogenously synthesized NO and exogenous NO appear to inhibit HIV replication. The data represent the mean of 5 duplicate cultures and are representative of three separate experiments.

Since NO produced endogenously by EBV-infected B cell lines inhibits EBV reactivation from a latent to a lytic state, the effect of endogenous NO synthesis on latent HIV infection was analyzed. The U-1 cell line (a promonocytic cell line) latently infected with HIV was grown in the presence or absence of L-NMA. The

effect on EBV reactivation was determined 2-3 days later by measuring p24 antigen levels in the cell supernatants.

Figure 4B shows the results when the latently infected U1 cell line was grown in the presence or absence of L-NMA and TNFα. and after 2-3 days in culture p24 antigen levels were measured in the cell supernatants by ELISA. L-NMA caused a dose dependent increase in p24 antigen levels with maximal effects seen at a concentration of 1mM (Figure 4B). The L-NMA-induced increase in p24 levels was abrogated by excess L-arginine but not D-arginine. These results suggest that endogenous NO synthesis inhibits HIV reactivation in a latently infected monocytic cell line. The data represent the mean of duplicate cultures and are representative of one of three similar experiments.

The actions of NO can be broadly classified as either cGMP dependent or independent. For example. much of the inhibitory effect of NO on platelets and smooth muscle is mediated by increases in cGMP levels due to activation of guanylate cyclase.

Figure 5 shows the effect of cGMP on HIV replication. Peripheral blood mononuclear cells were infected with HIV-1 and then grown in the presence or absence of SNAP. NAP or the cGMP analog 8-bromo-cGMP. After 5 days p24 antigen levels were measured in the cell supernatants. Thus. activation of guanylate cyclase may be one of the mechanisms by which NO inhibits HIV replication since 1mM 8-bromo-cGMP (a cGMP analog) caused a three-fold inhibition of HIV replication (Figure 5); however. other mechanisms may also be involved in the NO-induced inhibition of HIV replication since NO-generating compounds can decrease HIV replication 7 fold without significantly inhibiting cellular proliferation (150 uM). The data represent the mean of duplicate cultures.

The scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

What Is Claimed Is:

1. A method for inhibiting retroviral replication in a retrovirally infected cell or tissue culture *in vitro* which comprises contacting said retrovirally infected cell or tissue culture with an amount of nitric oxide or a nitric oxide-delivering, releasing or transferring compound sufficient to inhibit retroviral replication in said retrovirally infected cell or tissue culture.

2. The method of claim 1 which comprises administering gaseous nitric oxide.

3. A method for inhibiting retroviral replication in a retrovirally infected animal which comprises administering to said retrovirally infected animal an amount of nitric oxide or a nitric oxide-delivering, releasing or transferring compound sufficient to inhibit retroviral replication in said retrovirally infected animal.

4. The method of claim 2 which comprises administering a nitric oxide delivering, releasing or transferring compound selected from the group consisting of S-nitrosothiols, compounds that include at least one -O-NO group, N-nitrosoamines. C-nitroso compounds including at least one -C-NO group, nitrates having at least one $-O-NO_2$ group, nitroso-metal compounds, N-oxo-N-nitrosoamines, and thionitrates.

5. The method of claim 4 wherein the compound is an S-nitrosothiol.

6. The method of Claim 5 wherein the S-nitrosothiol is selected from the group consisting of an S-nitroso-amino acid, an S-nitroso-polypeptide and an S-nitrosylated protein.

7. The method of claim 5 wherein the S-nitrosothiol is selected from the group consisting of those having the structures:

(*i*) $CH_3(CH_2)_xSNO$

wherein x equals 2 to 20;

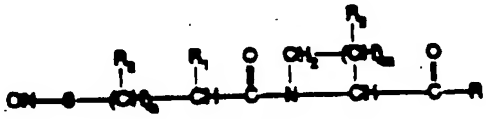
(*ii*) $HS(CH_2)_xSNO$

wherein x equals 2 to 20; and

(*iii*) $ONS(CH_2)_xY$

wherein x equals 2 to 20 and Y is selected from the group consisting of fluoro, C_1-C_6 alkoxy, cyano, carboxamido, C_3-C_6 cycloalkyl, aralkoxy, C_2-C_6 alkylsulfinyl, arylthio, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl; wherein aryl includes benzyl, naphthyl, and anthracenyl groups.

8. The method of claim 5 wherein the S-nitrosothiol is an S-nitroso-ACE inhibitor selected from the group consisting of compounds having the following structure (1):



(1)

wherein

R is hydroxy, NH_2, NHR^4, NR^4R^5, or C_1-C_7 alkoxy, wherein R^4 and R^5 are C_1-C_4 alkyl, or phenyl, or C_1-C_4 alkyl substituted by phenyl;

R^1 is hydrogen, C_1-C_7 alkyl, or C_1-C_7 alkyl substituted by phenyl, amino, guanidino, NHR^6, NR^6R^7, wherein R^6 and R^7 are methyl or C_1-C_4 alkanoyl;

R^2 is hydrogen, hydroxy, C_1-C_4 alkoxy, phenoxy, or C_1-C_7 alkyl;

R^3 is hydrogen, C_1-C_4 or C_1-C_7 alkyl substituted by phenyl;

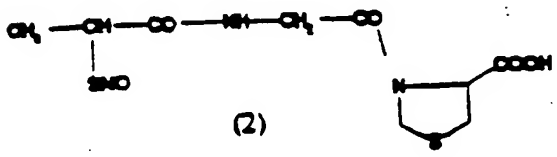
m is 1 to 3; and

n is 0 to 2.

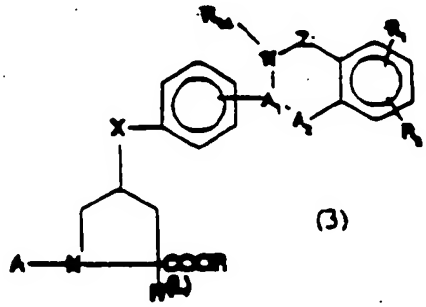
9. The method of claim 5 wherein the S-nitrosothiol is an S-nitroso-ACE inhibitor selected from the group consisting of N-acetyl-S-nitroso-D-cysteinyl-L-proline, N-acetyl-S-nitroso-D,L-cysteinyl-L-proline, 1-[4-amino-2-(S-nitroso)mercaptomethyl butanoyl]-L-proline, 1-[2-hexanoyl]-L-proline, 1-[5-guanidino-2-(S-nitroso)mercaptomethyl-pentanoyl]-L-proline, 1-

[5-amino-2-(S-nitroso) mercaptomethyl-pentanoyl]-4-hydroxy-L-proline, 1-[5-guanidino-2-(S-nitroso)mercaptomethyl-pentanoyl]-4-hydroxy-L-proline, 1-[2-aminomethyl-3(S-nitroso)-mercaptomethyl-pentanoyl-L-proline, and S-nitroso-L-cysteinyl-L-proline.

10. The method of claim 5 wherein the S-nitrosothiol is an S-nitroso-ACE inhibitor selected from the group consisting of compounds having structures (2-3):



(2)



(3)

wherein

X is oxygen or sulfur;

$-A_1,-A_2-$ is CH-NH or -C=N-;

R_3 0

A is ON-S-CH_2-CH-C;

R is selected from hydrogen, lower (C_1-C_4) alkyl, benzyl, benzhydryl, and salt forming ion;

R_1 and R_2 are independently selected from hydrogen, halogen, lower alkyl, lower alkoxy, halo substituted lower alkyl, nitro, and SO_2NH_2;

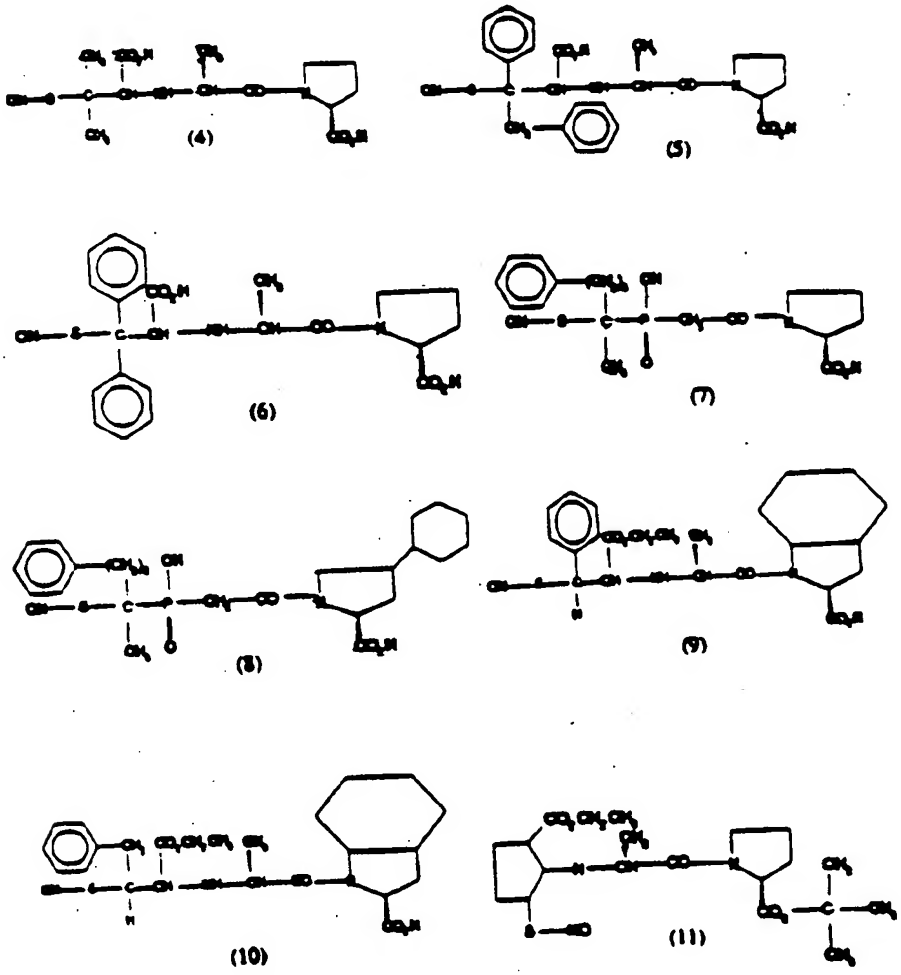
0 0 0

Z is -C- or -S-

R_3 is hydrogen, lower alkyl, halo substituted lower alkyl, phenyl, benzyl, phenethyl, or cycloalkyl; and

R_4 is hydrogen, lower alkyl, halo substituted lower alkyl, hydroxy substituted lower alkyl, $-(CH_2)_q-N$ (lower alkyl)$_2$ or $-(CH_2)_q-NH_2$ and q is one, two, three or four.

11. The method of claim 5 wherein the S-nitrosothiol is an S nitroso-ACE inhibitor selected from the group consisting of compounds having structures (4-11):



12. The method of claim 3 wherein the retrovirus is selected from the group consisting of human immunodeficiency virus and human T-cell leukemia virus-1.

13. The method of claim 3 wherein the animal is a human.

14. The method of claim 1 wherein the retrovirus is selected from the group consisting of human immunodeficiency virus and human T-cell leukemia virus-1.

15. A method for inhibiting the reactivation of latent retrovirus in a retrovirally infected cell or tissue culture which comprises contacting said retrovirally infected cell or tissue culture with a retrovirus reactivation inhibiting amount of nitric oxide, or a nitric oxide releasing, donating, or transferring substance may be used prophylactically to prevent reactivation of latent retrovirus.

16. A method for inhibiting the reactivation of a latent retrovirus infection in an animal in need thereof which comprises administering to said animal in need thereof an amount of nitric oxide, or a nitric oxide releasing, donating, or transferring substance sufficient to inhibit reactivation of latent retrovirus in said animal.

17. The method of claim 16 wherein the animal is a human.

18. A method for preventing or reversing retroviral latency in a retrovirally infected cell or tissue culture *in vitro* which comprises contacting a retrovirally infected cell or tissue culture *in vitro* with a nitric oxide synthase inhibitor or nitric oxide scavenger in an amount sufficient to render the latent retrovirus in the retrovirally infected cell or tissue culture replication-competent.

19. A method for the treatment of a latent retrovirus infection in an animal infected with a latent retrovirus infection which comprises administering thereto (i) a nitric oxide synthase inhibitor or nitric oxide scavenger in an amount sufficient to render the retrovirus

infecting said animal replication-competent and (ii) a retroviral replication inhibitory amount of an antiviral agent.

20. The method of claim 19 wherein the nitric oxide synthase inhibitor is nitro-arginine.

21. The method of claim 20 wherein the nitric oxide synthase inhibitor is selected from the group consisting of N^G-monomethyl-L-arginine, diphenylene iodonium and related iodonium derivatives, N-nitro-L-arginine methyl ester, N-methyl-L-arginine, N-amino-L-arginine, ornithine, N-imino-ethyl-L-ornithine and ornithine derivatives, N-nitro-L-arginine, methylene blue, trifluoropiperazine, calcinarin, calmadulin binders, and methotrexate.

22. The method of claim 19 wherein the antiviral agent is a purine nucleoside analog.

23. The method of claim 22 wherein the purine nucleoside analog is selected from the group consisting of acyclovir and didanosine.

24. The method of claim 19 wherein the antiviral agent is a pyrimidine nucleoside analog.

25. The method of claim 24 wherein the pyrimidine nucleoside analog is selected from the group consisting of zidovudine, trifluridine and zalcitabine.

26. The method of claim 19 wherein the antiviral agent is selected from the group consisting of a chelating agent, an intercalating agent and an interferon.

27. The method of claim 19 wherein the animal is a human.

28. A composition for the treatment of a latent retrovirus infection in an animal infected with a latent retrovirus infection which comprises (i) a nitric oxide synthase inhibitor or nitric oxide scavenger in an amount sufficient to render the retrovirus infecting said animal replication competent and (ii) a retrovirus replication inhibitory amount of an antiviral agent in a pharmaceutically acceptable carrier.

29. The composition of claim 28 wherein the nitric oxide synthase inhibitor is nitro-arginine.

30. The composition of claim 29 wherein the nitric oxide synthase inhibitor is selected from the group consisting of N^G-monomethyl-L-arginine, diphenylene iodonium and related iodonium derivatives, N-nitro-L-arginine methyl ester, N-methyl-L-arginine, N-amino-L-arginine, ornithine, N-imino-ethyl-L-ornithine and ornithine derivatives, N-nitro-L-arginine, methylene blue, trifluoropiperazine, calcinarin, calmadulin binders, and methotrexate.

31. The composition of claim 28 wherein the antiviral agent is a purine nucleoside analog.

32. The composition of claim 31 wherein the purine nucleoside analog is selected from the group consisting of acyclovir and didanosine.

33. The composition of claim 28 wherein the antiviral agent is a pyrimidine nucleoside analog.

34. The composition of claim 33 wherein the pyrimidine nucleoside analog is selected from the group consisting of zidovudine, trifluridine and zalcitabine.

35. The composition of claim 28 wherein the antiviral agent is selected from the group consisting of a chelating agent, an intercalating agent and an interferon.

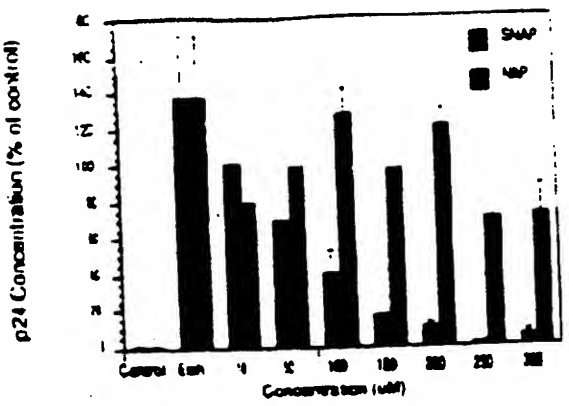


Figure 1A

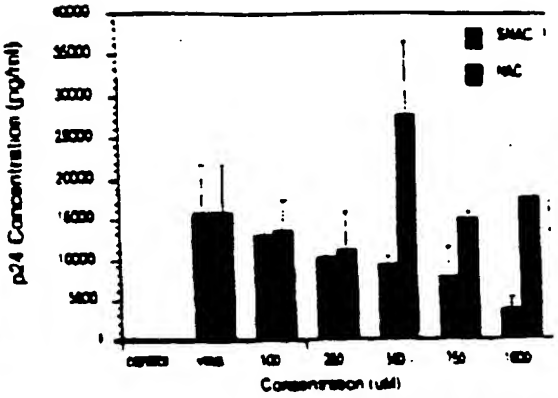


Figure 1B

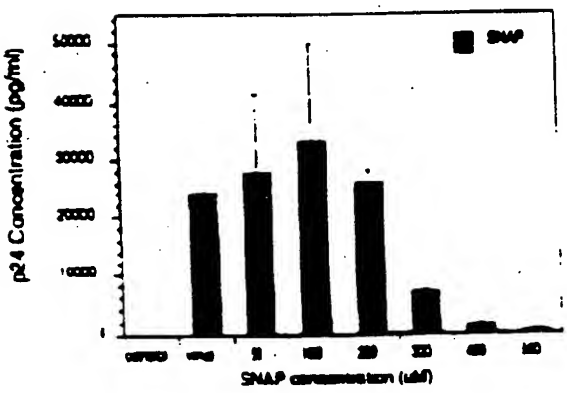


Figure 1C

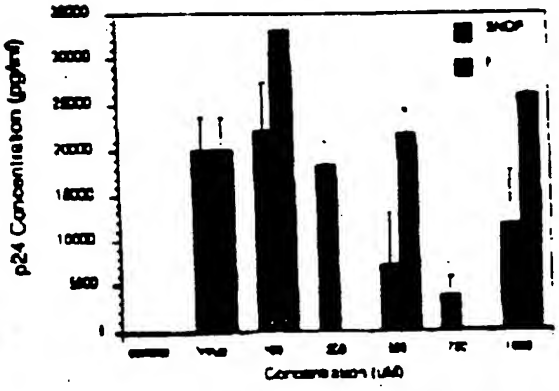


Figure 1D

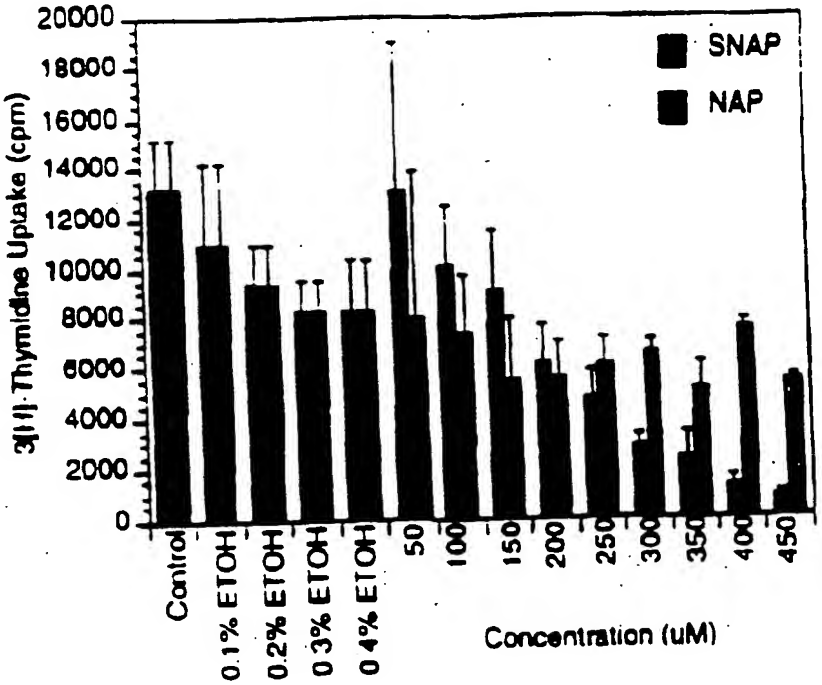


Figure 2A

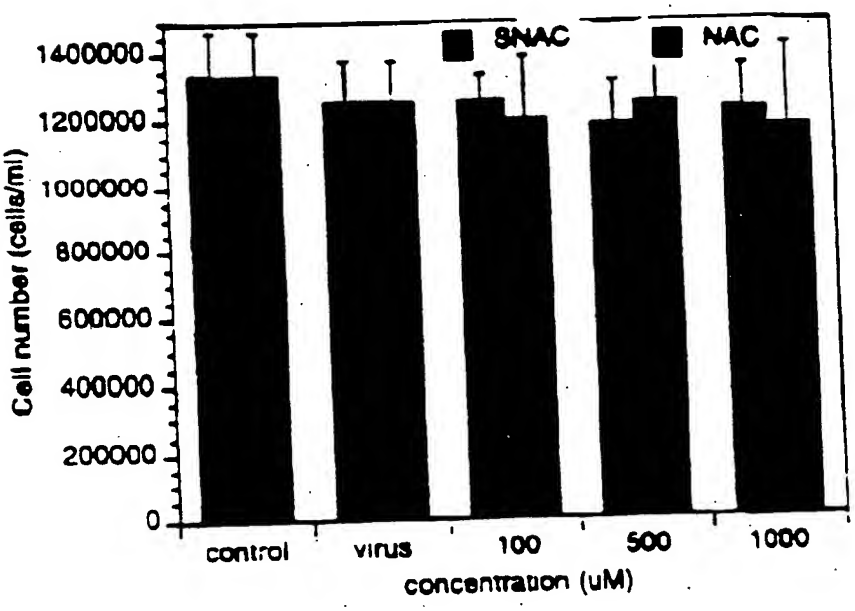


Figure 2B

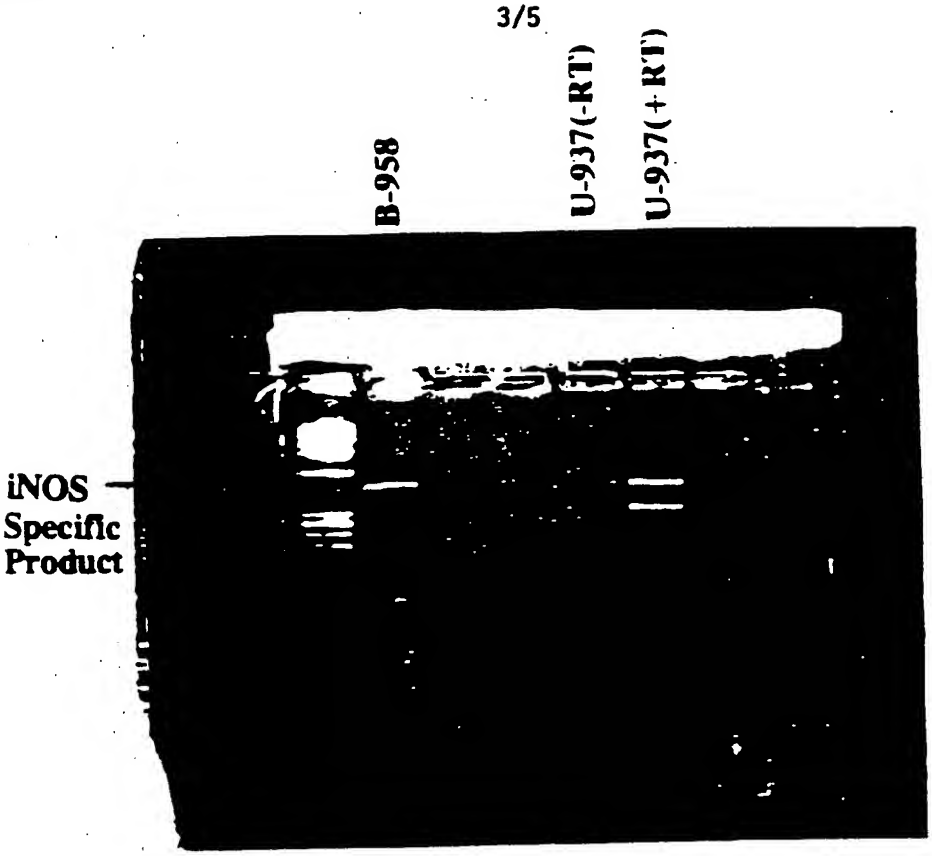


Figure 3A

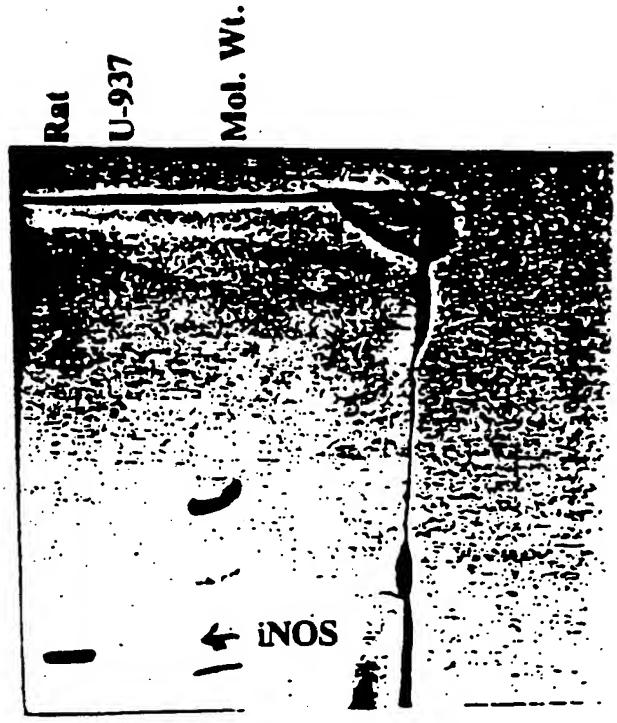


Figure 3B

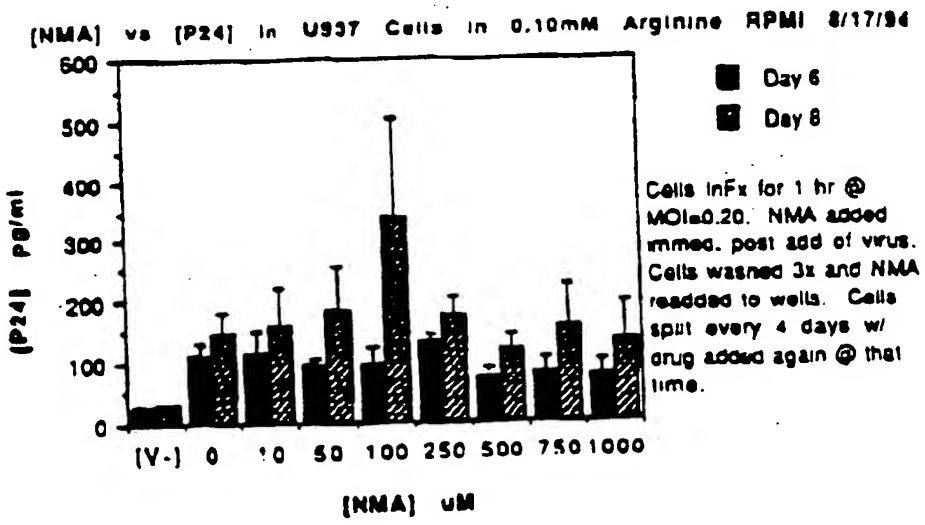


Figure 4A

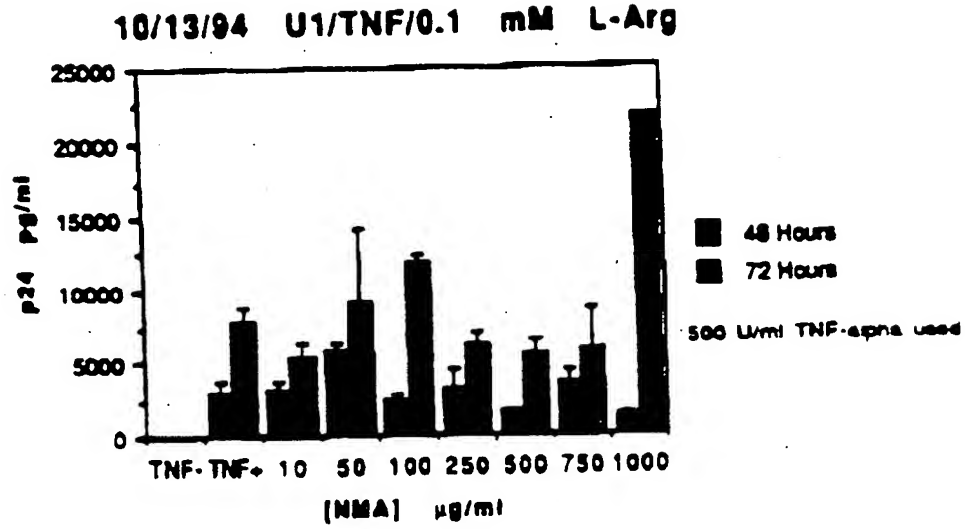


Figure 4B

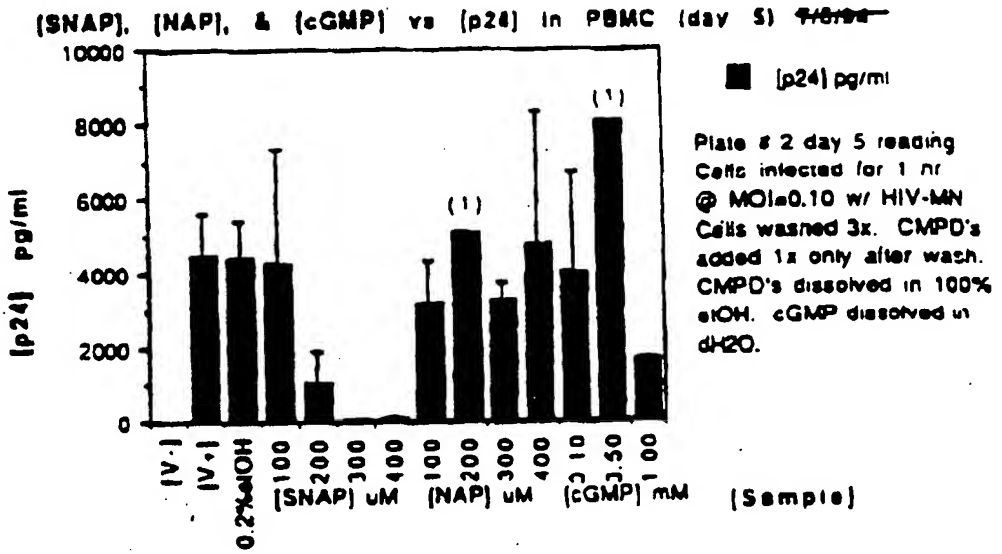

[SNAP], [NAP], & [cGMP] vs [p24] in PBMC (day 5)
[p24] pg/ml
10000
8000
6000
4000
2000
0
[p24] pg/ml
(1)
(1)
[p24] pg/ml
Plate # 2 day 5 reading
Cells infected for 1 hr
@ MOI=0.10 w/ HIV-MN
Cells washed 3x. CMPD's
added 1x only after wash.
CMPD's dissolved in 100%
etOH. cGMP dissolved in
dH2O.
[V-]
[V+]
0.2%etOH
100
200
300
400
100
200
300
400
10
50
100
[SNAP] uM
[NAP] uM
[cGMP] mM
[Sample]

Figure 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/04084

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/675, 31/40, 31/195
US CL :514/91, 423, 562
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/91, 423, 562

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN COMPOUNDS AND ANTIVIRAL MEHODS OF USE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Vol 261, issued 10 September 1993, Karupiah et al, "Inhibition of Viral Replication by Interferon-delta induced Nitric Oxide Synthase", pages 1445-48, entire document.	1-35
Y	US,A, 5,002,964 (LOSCALSO) 26 March 1991, entire document.	1-35
Y	US,A, 5,380,758 (STAMLER ET AL) 10 January 1995, entire doucment.	1-35
Y	US,A, 5,025,001 (LOSCALSO ET AL) 18 June 1991, entire document.	1-35
Y	US,A, 5,047,407 (BELLEAU ET AL) 10 September 1991, entire document.	28-35

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
25 JULY 1995

Date of mailing of the international search report
27 AUG 1996

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